REMARKS

In the Action, claims 13-19 are rejected. In response claims 13-19 are amended and new claims 25 and 26 are added.

Claims 13 and 17 are amended to delete the reference to the R_2 group being acetylenic and the methyl branches. The claims are further amended to recite a pharmaceutical composition comprising the claimed compound and to include a pharmaceutically acceptable excipient in the form of an oral composition. Claim 17 is also amended to correct the clerical error note in the Action. These amendments are supported by the specification as originally filed.

New claims 20 and 21 are added to recite a method of treating hypercholesterolaemia by administering the composition. Support for these claims is found on page 8 of the specification.

In view of these amendments and the above comments, reconsideration and allowance are requested.

The Rejections

Claims 13-16 are rejected as being anticipated by CS 212049 to Kocian. Kocian is cited for disclosing a single compound within the scope of the formula of claim 13. As amended the claims are not anticipated. Kocian does not disclose or suggest a pharmaceutical composition comprising a compound as now recited in combination with a pharmaceutical excipient in an oral dosage form. Kocian relates only to plant growth promoting compounds and not pharmaceutical compositions. Accordingly claim 13 is not anticipated by Kocian.

Claim 14-16 are also not anticipated as depending from an allowable base claim and for reciting additional features of the invention. Kocian does not disclose the claimed composition where R_1 has 7-13 carbon atoms and R_2 has 10-20 carbon atoms. Kocian also

does not disclose the composition where R_1 is a saturated linear C_9 carbon chain as in claim 15 or where R_2 is a hydrocarbon chain of a fatty acid as in claim 16.

Claims 13, 14 and 16 are rejected as being anticipated by U.S. Patent No. 6,656,662 to Okawa et al. Applicants respectfully submit that Okawa "662 is not a reference in this application. Section 102(e) states that a patent resulting from an international application filed before November 29, 2000 shall not be effective as prior art as of the international filing date. However, such patents shall be effective as prior art in accordance with 102(e) in effect on November 29, 2000. Under section 102(e) an application filed from an international application is prior art only if it was filed in English and designated the United States.

Notwithstanding, Okawa does not anticipate the claims as amended since Okawa does not disclose or suggest the claimed compounds. The Action refers to Okawa as disclosing a compound where R_1 is 8 and R_2 is 18. However, it is not clear where Okawa discloses this feature as indicated in the Action. Column 3, lines 38-49 disclose a compound where the group corresponding to R_1 is 9 and the group corresponding to R_2 is an acetylene-group containing hydrocarbon chain having 13 carbon atoms.

Furthermore, Okawa is specifically directed to a diacetylene compound having two adjacent acetylene (triple bond) groups. The compounds of Okawa are directed to polymerizable compounds the undergo polymerization by ultraviolet light. This has no relation to the claimed invention as recited in the amended claims.

In contrast to Okawa, the claimed compounds of claim 13 include an ethylenically unsaturated double bond between the R_1 and R_2 groups. Okawa does not disclose or suggest the claimed ethylenically unsaturated bond. The compounds disclosed in Okawa do not fall within the scope of the claim 13. For example, Okawa identifies "10,12-pentacosadienic acid" in column 3, lines 45-46. However, this compound does not fall within the scope of the

formula recited in claim 13. Accordingly, claim 13 and dependent claims 14 and 16 are not anticipated.

Claims 13, 14 and 16-19 are also rejected as being obvious over Okawa or Kocian in view of the disclosure in the specification or U.S. Patent No. 6,328,998 to Cavazza. The rejection is based on the position that it would have been obvious to include the claimed compounds in a composition.

For the reasons discussed above, Kocian and Okawa do not disclose or suggest the claimed compounds or the claimed composition. Furthermore, applicant's specification does not provide the suggestion or motivation to use the claimed compounds in the claimed composition. Pages 7-9 of the specification referred to in the Action clearly describe applicant's invention and is not prior art. It is improper to use applicant's disclosure of the invention as a basis for obviousness. Accordingly, the Action fails to establish prima facie obviousness.

Cavazza relates only to saturated compounds, such as octacosanol. There is no suggestion of the claimed unsaturated compounds of the present invention or the compounds disclosed in Kocian and/or Okawa. Furthermore, it would not have been obvious to one of ordinary skill in the art to produce an oral composition using the plant growth compounds of Kocian or the polymerizable compounds of Okawa. Accordingly, the claims are not obvious over the art of record.

Applicants have found that the claimed compounds exhibit a hypocholesterolemic effect compared to other unsaturated fatty acids. Specifically, applicants have found the compound of the invention octacosenol exhibits a hypocholesterolemic effect that is not found in compounds outside the scope of the claimed invention. For example, EPA (eicosapentaenoic acid) and DHA (docohesanoic acid) which are highly unsaturated with 20 and 22 carbon atoms respectively do not exhibit the same hypocholesterolemic effect and

show a reduction of triglyceride levels, but no effect on serum cholesterol levels. See for example the Attachment A which discloses that the effects of EPA and DHA are known.

Also enclosed is an experimental report by the inventors (Attachment B), which shows the effects of the present invention. These effects would not be expected by one skilled in the art. Accordingly, it would not have been obvious to one skilled in the art to use the plant growth compounds of Kocian or the polymerizable compound of Okawa in an oral composition.

In view of the above comments and these amendments, the claims are submitted to in condition for allowance. Reconsideration and allowance are requested.

Respectfully submitted,

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The impact of EPA and DHA on blood lipids and lipoprotein metabolism: influence of apoE genotype. Anil, Eliz. Hugh Sinclair Unit of Human Nutrition, Department of Food Biosciences, University of Reading, Reading, UK. Proceedings of the Nutrition Society (2007), 66(1), 60-68. Publisher: Cambridge University Press, CODEN: PNUSA4 ISSN: 0029-6651. Journal; General Review written in English. CAN 146:481201 AN 2007:473557

Abstract

A review. Fish and fish oil-rich sources of long-chain n-3 fatty acids have been shown to be cardio-protective, through a multitude of different pathways including effects on arrythymias, endothelial function, inflammation and thrombosis, as well as modulation of both the fasting and postprandial blood lipid profile. To date the majority of studies have examd. the impact of EPA and DHA fed simultaneously as fish or fish oil supplements. However, a no. of recent studies have compared the relative biopotency of EPA v. DHA in relation to their effect on blood lipid levels. Although many beneficial effects of fish oils have been demonstrated, concern exists about the potential deleterious impact of EPA and DHA on LDL-cholesterol, with a highly-heterogenous response of this lipid fraction reported in the literature. Recent evidence suggests that apoE genotype may be in part responsible. In the present review the impact of EPA and DHA on cardiovascular risk and the blood lipoprotein profile will be considered, with a focus on the apoE gene locus as a possible determinant of lipid responsiveness to fish oil intervention.

Omega-3 fatty acids from fish oils and cardiovascular disease. Holub, Darren J.; Holub, Bruce J. Department of Psychiatry and Behavioural Neurosciences, Faculty of Health Sciences, McMaster University, Hamilton, ON, Can. Molecular and Cellular Biochemistry (2004), 263(1&2), 217-225. Publisher: Kluwer Academic Publishers, CODEN: MCBIB8 ISSN: 0300-8177. Journal; General Review written in English. CAN 142:73839 AN 2004:834826

Abstract

A review. Fish and fish oils contain the ω-3 fatty acids known as eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA). Epidemiol. studies have shown an inverse relation between the dietary consumption of fish contg. EPA/DHA and mortality from coronary heart disease. These relationships were substantiated from blood measures of ω-3 fatty acids including DHA as a physiol. biomarker for ω-3 fatty acid status. Controlled intervention trials with fish oil supplements enriched in EPA/DHA have shown their potential to reduce mortality in post-myocardial infarction patients with a substantial redn. in the risk of sudden cardiac death. The cardioprotective effects of EPA/DHA are widespread, appear to act independently of blood cholesterol redn., and are mediated by diverse mechanisms. Their overall effects include anti-arrhythmic, blood triglyceride-lowering, anti-thrombotic, anti-inflammatory, endothelial relaxation, plus others. Current dietary intakes of EPA/DHA in North America and elsewhere are well below those recommended by the American Heart Assocn. for the management of patients with coronary heart disease.

EXPERIMENTAL REPORT

HYPOCHOLESTEROLEMIC ACTIVITY

This study aims at comparing the hypocholesterolemic activity of octasenol, which is a compound according to the invention claimed in the European patent application no. 03732804, with that of the prior art unsaturated fatty acids EPA (eicosapentaenoic acid) and DHA (docosaenoic acid) in an animal model in which hypercholesterolemia is induced (i.e., the hypercholesterolemic rabbit).

The study was carried out on New Zealand white male rabbits (Harlan Italy, Correzzana) having a weight between 1 and 1.3 kg. The animals were housed in a controlled environment (cycle light/dark of 12 hours, 60% relative humidity at a temperature of 22°C) and fed with standard Harlan diet and water *ad libitum*.

After one week, the rabbits were weighted and random distributed in the different groups of treatment and control as shown in table 1. Blood samples from all the animals were taken from the marginal vein of the ear and the blood levels of cholesterol, triglycerides, HDL cholesterol and LDL cholesterol were measured ("time 0" - table 2).

Table 1 - EXPERIMENTAL GROUPS

Group	No. of rabbits	Dose/die (mg/kg)	Type of treatment
negative control	5	-	standard diet
positive control	5	-	hypercholesterolemic diet (D.IP.)
OLO	5	50	D.IP. + Octasenol
EPA	5	50	D.IP. + EPA
DHA	5	50	D.IP. + DHA
EPA + DHA	5	50	D.IP. + EPA + DHA
EPA	5	500	D.IP. + EPA
DHA	5	500	D.IP. + DHA
EPA + DHA	5	500	D.IP. + EPA + DHA

The hypercholesterolemic diet was formulated according to Menendez et al. (1997) and described by Kroon et al. (1982) and the relevant composition is shown in figure 1.

For all of the experimental groups, the diet was limited to the amount of 100g/die, according to these Authors.

The animals of the negative control group were fed with the standard diet; those of the positive control group and those of the OLO, EPA, DHA and EPA + DHA groups were fed with the hypercholesterolemic diet (D.IP.).

In parallel, the animals of the OLO, EPA, DHA and EPA + DHA groups were daily treated for 60 consecutive days with the mentioned substances. The compounds under examination were administered *per os* through a specific intragastic cannula at a dose of 50 mg/kg body weight and, limited to EPA, DHA and EPA + DHA, at a dose of 500 mg/kg body weight, in the form of a water/Tween 20 suspension (administered volume per animal per day: 1 ml/kg body weight/day) (Menendez et al., 1997). The animals belonging to the negative control and positive control groups received an equal volume (1 ml/kg body weight/day) of a water/Tween 20 mixture.

After 60 days of treatment, blood samples were taken from each animal from the external marginal vein of the ear. The blood samples were tested to measure the blood levels of total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol, and the effect of the various types of treatments on platelet aggregation (table 4).

The rabbits, after having been weighted (table 3), were killed according national and international guidelines (D.L. no. 116, G.U. Suppl. 40, February 18, 1992, Circolare no. 8, G.U. July 1994; EEC Council Directive 86/609, OJL 358, 1 December 12, 1987; guide for the care and Use of Laboratory Animals published by the US National Institute of health - NIH Publication no. 85-23, revised 1996). The rabbits were killed by i.p. injection of sodium pentobarbital (50 mg/ml) and the aortas were taken (from the exit of the left ventricle up to about 5 mm after the iliac bifurcation) and placed into a 4% formalin solution for the analysis of the presence or absence of atherosclerotic plaques.

Table 2 - BASAL HEMATOCHEMICAL PARAMETERS BEFORE TREATMENT ("time 0")

Experimental group	Cholesterol (mmol/L)	Triglycerids (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
NEG CTRL	1.75±0,42	1.55±0,40	0,75±0,24	0,57±0,20
POS CTRL	1.72±0,33	1.49±0.25	0.64±0.21	0.63±0.17
OLO (50 mg/kg)	1.87±0.45	1.59±0.39	0.74±0.26	0.54±0.19
EPA (50 mg/kg)	1.70±0.25	1.60±0.27	0.66±0.30	0.56±0.22
DHA (50 mg/kg)	1.84±0.39	1.59±0.35	0.69±0.25	0.51±0.16
EPA+DHA (50 mg/kg)	1.71±0.44	1.53±0.38	0.79±0.22	0.61±0.19
EPA (500 mg/kg)	1.72±0.41	1.54±0.26	0.64±0.41	0.54±0.22
DHA (500 mg/kg)	1.75±0.33	1.49±0.34	0.68±0.26	0.60±0.28
EPA+DHA (500 mg/kg)	1.74±0.29	1.57±0.26	0.65±0.32	0.59±0.25

Table 3 - EFFECT OF THE VARIOUS TREATMENTS ON THE ANIMAL WEIGHT

Experimental group	time 0 (Kg)	time 60 (Kg)
NEG CTRL	1.2±0.4	2.2±0.3
POS CTRL	1.1±0.3	1.9±0.1
OLO (50 mg/kg)	1.1±0.1	2.0±0.3
EPA (50 mg/kg)	1.2±0.1	2.3±0.4
DHA (50 mg/kg)	1.2±0.3	2.1±0.3
EPA+DHA (50 mg/kg)	1.1±0.2	2.2±0.1
EPA (500 mg/kg)	1.2±0.1	2.0±0.2
DHA (500 mg/kg)	1.0±0.3	1.8±0.2
EPA+DHA (500 mg/kg)	1.3±0.1	2.1±0.3

Table 4 - EFFECT OF THE COMPOUNDS UNDER EXAMINATION ON SOME HEMATOCHEMICAL PARAMETERS AFTER DAILY ADMINISTRATION *PER OS* FOR 60 CONSECUTIVE DAYS TO THE RABBIT

Experimental group	**Cholesterol (mmol/L)**	**Triglycerids (mmol/L)**	**HDL (mmol/L)**	**LDL (mmol/L)**	**Platelet aggregation[1] (aggregation %)**
NEG CTRL	1.80±0.25	1.59±0.46	0.65±0.28	0.58±0.27	20.4±3.9
POS CTRL	4.16±1.02[a]	1.75±0.39	0.71±0.27	0.89±0.34[a]	25.6±4.9
OLO (50 mg/kg)	2.4±0.89[b]	1.68±0.73	0.81±0.21[a,b]	0.63±0.38[b]	25.6±2.3
EPA (50 mg/kg)	3.85±1.14	1.54±0.85	0.69±0.27	0.78±0.39	23.1±2.9
DHA (50 mg/kg)	3.72±0.97	1.53±0.91	0.73±0.24	0.73±0.21	23.9±4.0
EPA+DHA (50 mg/kg)	3.67±0.83	1.43±0.64	0.70±0.29	0.75±0.31	23.2±3.8
EPA (500 mg/kg)	3.81±0.34	1.50±0.53[a]	0.65±0.22	0.74±0.29	23.5±2.4
DHA (500 mg/kg)	3.95±0.26	1.49±0.34 [a]	0.69±0.32	0.69±0.21	24.3±2.6
EPA+DHA (500 mg/kg)	3.79±0.24	1.37±0.42 [a]	0.73±0.31	0.78±0.26	23.4±3.1

[1] ADP was used as the agonist (2.5 µmol/ml)

[a] $p<0.01$ over the negative control (Wilcoxon test)

[b] $p<0.05$ over the negative control (Wilcoxon test)

RESULTS AND DISCUSSION

The rabbits were weighted before the beginning of the treatment and after 60 days of treatment either with a standard diet or with a hypercholesterolemic diet with daily oral administration of the compounds under examination. The weight gain measured under the different conditions can be considered as comparable.

The hematochemical parameters which were taken into account in this study are the triglycerides, the total cholesterol and the HDL cholesterol and LDL cholesterol. The results obtained from the blood samples withdrawn before the administration of the compounds under examina-

tion, and particularly the results obtained from the positive control group, show that the total cholesterol levels are much lower than those reported by Menendez at al, 1997, though they are still higher than those of negative control group (standard diet). The other parameters (triglycerides, LDL and HDL) are almost the same as those reported in the literature.

Since the hypercholesterolemia in the animal model is induced with the diet, it is possible to detect an experimental variation which, however, is in agreement with the data reported in the literature (Rong J.X. et al., 1999; Bode-Böger S.M. et al., 1998; de la Peña N.C. et al., 2000). Furthermore, it is to be taken into account that the values measured during this study show a high standard deviation, which is likely due to the low number of animals in each group.

After 60 days of treatment, the results obtained show that the daily administration of EPA or DHA or EPA + DHA at a dose of 50 mg/kg body weight and at a dose of 500 mg/kg body weight, in combination with a hypercholesterolemic diet, leads to decreased triglycerides levels compared with the negative control, whilst the cholesterol levels remain unchanged. On the contrary, the daily administration of octasenol (OLO) in combination with a hypercholesterolemic diet leads to a statistical significant decrease in the cholesterol levels, leaving the triglycerides and LDL levels substantially unchanged. In contrast, the HDL levels are significantly increased compared to the controls. These results are comparable with the results obtained in previous studies which showed the protective effects of the EPA and DHA fatty acids contained in omega-3 PUFAs towards cardiovascular diseases (Saldeen T. et al., 2001), which are due to a decrease in the triglycerides levels. The administration of EPA and DHA improves insulin activity in peripheral tissues in diabetes mellitus patients, thereby reducing other risk factors such as the plasma concentration of triglycerides and hypertension (Berry et al., 1997; Kasim-Karakas et al., 2001).

Both the positive control group (D.IP. alone) and the groups fed with D.IP. and treated with the compounds under examination, were negative for the presence of atherosclerotic plaques. The pathologist contacted suggested that the blood cholesterol levels observed (which were slightly higher than twice the negative control) do not justify the onset of atherosclerotic plaques: the absence of plaques is therefore in accordance with the results obtained.

Taken as a whole, the results obtained show an hypocholesterolemic effect in the group treated with OLO and an hypotriglycemizing effect in the groups treated with different doses

of EPA and/or DHA.

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EXPERIMENTAL REPORT

HYPOCHOLESTEROLEMIC ACTIVITY

The study aims at analysing the hypocholesterolemic activity of the compounds octacosenol and octacosenoic acid compared with that of commercial polycosanols of natural origin.

The study has been carried out on New Zealand white male rabbits (Harlan Italy, Correzzana) having a weight between 1 and 1.3 kg. The animals were kept in a controlled environment (cycle light/dark of 12 hours, relative humidity 60% and temperature of 22°C) and fed with standard Harlan diet and water *ad libitum*.

The rabbits were weighed and random distributed in the different groups of treatment and control as shown in table 1. After one week, samples of blood from all the animals were taken from the external marginal vein of the ear and the levels in blood of cholesterol, triglycerides, HDL cholesterol and LDL cholesterol were measured ("time 0" – table 2).

Table 1 – EXPERIMENTAL GROUPS

Group	Number of rabbits	Type of treatment
Negative control	4	Standard diet
Positive control	4	Hypercholesterolemic diet (D.IP.)
PSIIT	4	D.IP. + Polycosanols S.I.I.T.
PGC	4	D.IP. + Polycosanols Giellepi Chemicals
OLO	6	D.IP. + Octacosenol
OICO	6	D.IP. + Octacosenoic acid

The hypercholesterolemic diet was formulated according to Menendez et al. (1997) and described by Kroon et al. (1982).

For all the experimental groups, the diet was limited to the amount of 100 g/die, according to the mentioned authors.

The animals relating to the negative control group were fed with the standard diet; those of the positive control group and those of groups PSIIT, PGC, OLO and OICO with hypocholesterolemic diet (D.IP.).

In parallel, the animals of the groups PSIIT, PGC, OLO and OICO were daily treated for 30 consecutive days with the same substances. The compounds under examination were administered *per os* by means of a specific intragastric cannula with the dosage of 50 mg/kg of body weight, in the form of a suspension in water/Tween 20 (volume administered per animal per day: 1 ml/kg of body weight/day) (Menendez et al., 1997). The animals belonging to the negative control and positive control group received an equal volume (1 ml/kg b.wt./day) of the mixture water/Tween 20.

It is understood that the animals belonging to the PSIIT and PGC groups received an amount of polycosanols SIIT and polycosanols Giellepi Chemicals having a content of active agent corresponding to the amount of octacosenol and octacosenoic acid (the compounds under test).

After 30 days of treatment, blood samples were taken from the external marginal vein and the levels in blood of total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol were measured. The results are given in table 4.

The rabbits, after having been weighed, were killed according to national and international guidelines by injection (i.p.) of sodium pentobarbital (50 mg/ml) and the aortas were taken out (from the exit of left ventricle up to about 5 mm after the iliac bifurcation) and set in a 4% formalin solution for the analysis of the presence of atherosclerotic plaques.

Table 2 – HEMATOCHEMICAL PARAMETERS AT THE BEGINNING OF THE TREATMENT ("time 0")

EXPERIMENTAL GROUP	CHOLESTEROL mmol/L	TRIGLYICERIDES mmol/L	HDL mmol/L	LDL mmol/L
CTRL NEG[a]	1.79±0.41	1.57±0.43	0.72±0.22	0.55±0.19
CTRL POS[a]	1.71±0.35	1.46±0.22	0.61±0.19	0.65±0.14
PSIIT[a]	1.89±0.46	1.57±0.43	0.79±0.21	0.58±0.21
PGC[a]	1.70±0.22	1.67±0.30	0.65±0.27	0.51±0.20
OLO[b]	1.85±0.38	1.61±0.38	0.70±0.24	0.50±0.17
OICO[b]	1.73±0.46	1.50±0.35	0.81±0.21	0.63±0.18

Values are the average ± standard deviation of 4[a] or 6 [b] independent experiments

Table 3 – EFFECT OF DIFFERENT TREATMENTS ON THE WEIGHTS OF ANIMALS

EXPERIMENTAL GROUP	Time 0 kg	Time 30 days kg
CTRL NEG[a]	1.2±0.3	1.7±0.3
CTRL POS[a]	1.3±0.2	1.8±0.2
PSIIT[a]	1.1±0.2	1.6±0.3
PGC[a]	1.1±0.1	1.7±0.4
OLO[b]	1.2±0.2	1.7±0.5
OICO[b]	1.0±0.1	1.6±0.6

Values are the average ± standard deviation of 4[a] or 6 [b] independent experiments

Table 4 – EFFECT OF THE COMPOUNDS ON HEMATOCHEMICAL PARAMETERS

EXPERIMENTAL GROUP	CHOLESTEROL mmol/L	TRIGLYCERIDES mmol/L	HDL mmol/L	LDL mmol/L
CTRL NEG[a]	1.87±0.38	1.66±0.49	0.67±0.23	0.53±0.23
CTRL POS[a]	4.27±1.05[c]	1.72±0.38	0.78±0.22	0.86±0.38[c]
PSIIT[a]	3.20±0.96	1.80±0.71	0.73±0.26	0.77±0.32
PGC[a]	3.18±1.04	1.60±0.87	0.65±0.24	0.71±0.33
OLO[b]	2.72±0.83[d]	1.79±0.89	0.77±0.19	0.69±0.28[d]
OICO[b]	2.85±0.82[d]	1.74±0.70	0.70±0.25	0.70±0.26[d]

Values are the average ± standard deviation of 4[a] or 6[b] independent experiments
[1]: ADP has been used as agonist (2.5 µmol/ml)
[c]$p<0.01$ in respect of the negative control (Wilcoxon)
[d]$p<0.05$ in respect of the positive control (Wilcoxon)

The treatment under the different conditions does not produce specific variations on the weight of the animals: the increase of weight during the experiments (0.5-0.6 kg) is in fact comparable under the different experimental situations.

The data show A remarkable effect on the LDL cholesterol level. The search of atherosclerotic plaques was negative both in the positive control group and in the groups fed with D.IP. and with the compounds under examination: it is however suggested that the levels of blood cholesterols which were found (which are about two times the negative control in the four weeks) do not however justify the formation of atherosclerotic plaques: the absence of such plaques is therefore compatible with the results which are obtained.

The obtained results show that the compounds under test (compounds of the invention – OLO and OICO) have an improved hypocholesterolemic effect with respect to the commercial polycosanols.

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